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Light response of the chlorophyll fluorescence parameters and partitioning of absorbed light energy in wild type and *npq4* mutant of *Arabidopsis thaliana*

Zsuzsanna Várkonyi[1]*, Szilvia Bajkán, Gyula Váradi[2], Endre Lehoczki

Department of Botany and Botanical Garden, University of Szeged, Szeged, Hungary, [1]Institute of Plant Biology, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary, [2]Research Institute for Viticulture and Enology, Kecskemét, Hungary

ABSTRACT Photoprotective thermal dissipation of absorbed light energy is measured as non-photochemical quenching (NPQ) of chlorophyll fluorescence during exposure of plants to higher light intensities. We investigated the light response behavior of chlorophyll fluorescence and energy partitioning in PSII of *npq4* mutant of *Arabidopsis thaliana*, which is lacking of PsbS protein, to that of the wild type plants. We found that the PsbS protein appears to function not only in thermal dissipation in light via xanthophyll pigments, but also modified the allocation pattern of excitation energy absorbed in the PSII to PSII photochemistry (P), thermal dissipation in light (DL) and excess excitation (E).

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KEY WORDS

Arabidopsis
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energy allocation

Photosynthetic organisms are exposed to a wide range of light intensities in nature. Above a certain level photosynthesis is incapable of using all of the energy absorbed by the light-harvesting complexes (LHCs). To prevent the damage caused by excess excitation various photoprotective mechanisms are present in the thylakoid membrane. One of these mechanisms is thermal dissipation of the excess excitation energy within the LHCII (Demmig-Adams et al. 1996; Elrad et al. 2002)

Photoprotective thermal dissipation of absorbed light energy is measured as non-photochemical quenching (NPQ) of chlorophyll fluorescence during exposure of plants to higher light intensities. In wild-type *Arabidopsis* and other plants several processes can contribute to NPQ; the major component is ΔpH-dependent NPQ, called qE. Following the induction of qE by light, the alteration of ΔpH activates synthesis of zeaxanthin, through the xanthophyll cycle. The conformational changes in the structure of LHCII, especially in PsbS protein caused by binding of protons and zeaxanthin to PsbS induce quenching of excitation energy. The *NPQ4* gene encodes the PsbS protein what is a member of the chlorophyll a/b-binding, light harvesting complex family of proteins and binds zeaxanthin. Genetic analysis showed that the phenotype of *npq4-1* mutant plants was due to a single, nuclear mutation, which blocks qE that is detected in the wild type plants. The *npq4-1* mutant lacks the ΔpH- and zeaxanthin-dependent conformational change in the thylakoid membrane that is necessary for qE (Li et al. 2002).

In the present study, we compared the light response behavior of chlorophyll fluorescence and energy partitioning in PSII of *npq4* mutant of *Arabidopsis thaliana* to that of the wild type plants.

*Corresponding author. E-mail: photosynt@nucleus.szbk.u-szeged.hu

Materials and Methods

Plant material

Seeds were germinated and grown in commercial potting soil mixture in the growth chamber, with 12 h /12 h day/night regime, with corresponding temperature 25°C/22°C under an irradiance of 100 μmol m^{-2} s^{-1} photon flux density (PFD).

Chlorophyll fluorescence measurements

Chlorophyll fluorescence measurements were carried out in dark adapted leaves with a pulse amplitude modulated fluorometer (PAM 200 Walz, Germany). In the measurements the standard setting of Run 8 were used according to the PAM-200 handbook (Operating Manual, 1st Edition, 1997). Before start of the Run 8, initial fluorescence yield (Fo) and maximum fluorescence yield (Fm) were determined in 60 min dark adapted samples with a saturating pulse (3000 μmol m^{-2} s^{-1} PFD). After start of the Run 8, a 5 min preillumination at 210 μmol m^{-2} s^{-1} actinic light (AL) intensity was applied in order to allow light adaptation of the sample and activation of Calvin cycle enzymes. Then AL changed to 60 μmol m^{-2} s^{-1} PFD and increased every 5 min until 1250 μmol m^{-2} s^{-1} PFD has reached (10 steps). The quenched levels of maximum chlorophyll fluorescence (Fm') parameters were determined at each AL level by saturated pulses (3000 μmol m^{-2} s^{-1} PFD, of 1 s duration) applied to the leaves at the end of 10 s AL illumination. After the AL have been switched off, far-red light was exerted for the determination of the minimal (Fo') level of fluorescence for correct determination of qP and Fv'/Fm' at each light intensity. The terminology suggested by van Kooten and Snel (1990) was used. From measured parameters (Fo, Fm, Fo', Fm' and the steady state fluorescence level, Fs) the

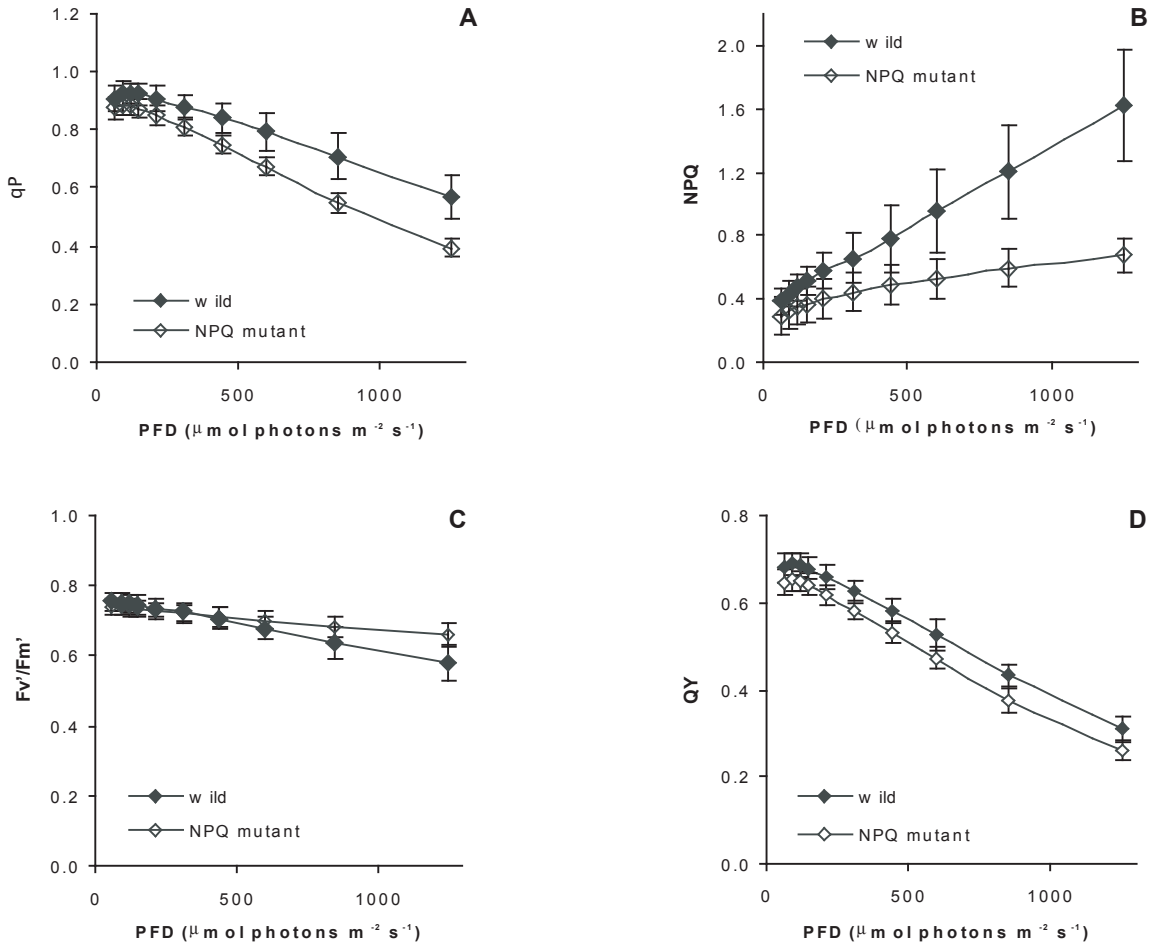

Figure 1. Actinic light response of the photochemical quenching (A), the yield of PSII photochemistry (B), the quantum yield of open PSII centers (C) and the non-photochemical quenching (D) in leaves of wild and *npq4* mutant of *A. thaliana*. The data are means ± SE of minimum 6 replicates from 3 independent experiments.

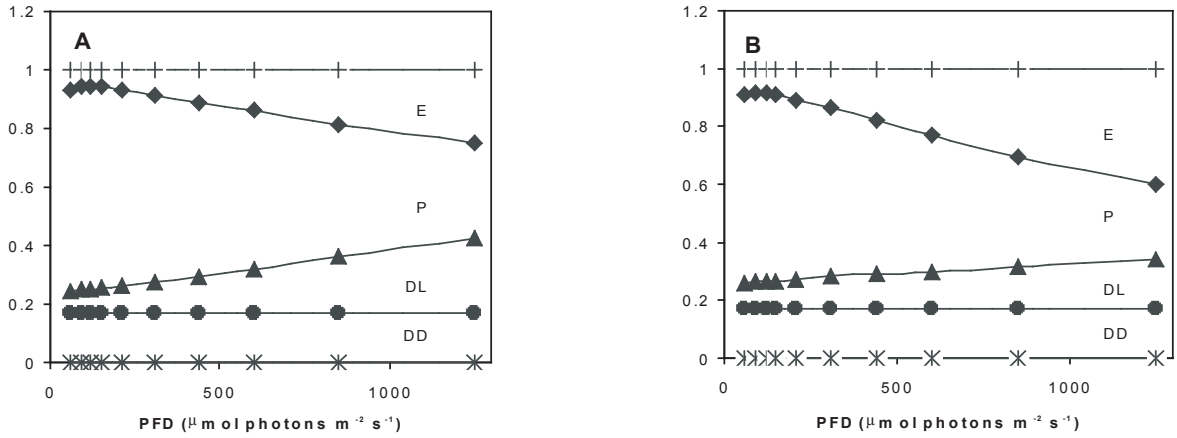

Figure 2. Light response allocation of light absorbed by the PSII antenna to PSII photosynthetic electron transport (area of P), thermal energy dissipation in the light (area of DL) and in the dark (area of DD), and to excess excitation (area of E) in the leaves of wild (A) and *npq4* mutant of *A. thaliana* (B). The data are calculated from the average values of the results presented in Figure 1.

Table 1. Pigment composition, sizes of the xanthophyll cycle pool and de-epoxidation index (DEi) of the leaves of wild and *npq4* mutant type of *Arabidopsis taliana*. The results are the means ± SE of the three independent experiments.

	Chl-a+b (μmol m^{-2})	Caroten. (μmol m^{-2})	Chl-a/b	Chl-a+b/ Carotenoids	V+A+Z (μmol m^{-2})	V+A+Z/Chl-a+Chl-b (μmol $mmol^{-1}$)	DEi
Wild	129 ± 4	41 ± 4	2,57 ± 0,1	3.2 ± 0,1	12 ± 1,6	93 ± 6	0,60 ± 0,03
Mutant	128 ± 3	46 ± 5	2,87 ± 0,2	2,8 ± 0,2	12,5 ± 1,1	98 ± 4	0,58 ± 0,03

optimum quantum yield (Fv/Fm), photochemical quenching coefficient (qP), non-photochemical quenching (NPQ) and relative quantum yield of PSII photochemistry (QY) were calculated according to Schreiber et al. 1986; Genty et al. 1989; Bilger and Björkman 1990 , respectively. The partitioning of absorbed light energy in PSII was calculated according to the model of Demmig-Adams et al. 1996)

Pigment analysis

Chlorophylls and total carotenoids were measured according to Lichtenthaler (1987). Xantophyll cycle components were determined by means of HPLC (Váradi et al. 1992).

Results and Discussion

In dark-adapted leaves the optimum quantum yield of PSII was 0,83±0,02 for both types of *A. thaliana*. Figure 1 shows the light response of calculated chlorophyll fluorescence parameters. The fraction of open PSII centers (qP, Fig. 1A), the quantum yield of PSII electron transport (QY, Fig. 1B) and the quantum yield of open PSII (Fv'/Fm', Fig. 1C) decreased with increasing PFD, but more sensitively with the light intensity in the *npq4* mutant plants. Only values of Fv'/Fm' at higher PFDs in mutant plant were slightly higher. Moreover, as expected, significantly lower level of NPQ was observed in *npq4* mutant of *A. thaliana* (Fig. 1D). However, under the growth conditions employed, pigment composition and the *in vivo* levels of the xanthophyll pigments violaxanthin (V), anteraxanthin (A) and zeaxanthin (Z) in excess light were the same for *npq4* mutant and wild type of *A. thaliana*, and showed very similar xanthophyll de-epoxidation (DEi) activity (Table 1).

For a better understanding the possible role of PsbS protein in the allocation of absorbed light energy to the PSII photochemistry and thermal dissipative processes was assessed for the wild and *npq4* mutant of *A. thaliana* with the model of Deming-Adams et al. (1996). The fraction of light absorbed by PSII antennae that is utilized in the PSII photochemistry was estimated from P=(Fv'/Fm') qP. Heat dissipation is defined as 1-Fv'/Fm', which was divided in to the fraction of energy dissipated in the dark (DD=(1-Fv/Fm)) and that dissipated in the light (DL=Fv/Fm-Fv'/Fm') via xanthophyll cycle. The fraction of absorbed light neither going into P, nor into DD and DL is defined as excess (E), and it is calculated as the product of (Fv'/Fm') (1-qP). The calculated energy partitioning is shown in Fig. 2. In both type of plants the fraction of photochemistry (P) decreased, but DL and E increased with increasing PFD. The *npq4* mutant showed slightly lower DL, significantly lower P, and considerably higher E values. It seems, that the decrease in DL and P contributed to the increase in excess energy, which is also a consequence of PsbS protein mutation.

The PsbS protein is necessary for photoprotective thermal dissipation of excess absorbed light energy in plants, measured as non-photochemical quenching of chlorophyll fluorescence. However, the ΔpH and xanthophyll cycle-dependent conformational change associated with qE is absent in *npq4* mutant. It is well-known that *npq4* mutant of *A. thaliana* that lack qE is more sensitive to light stress. Our results suggest that the PsbS protein appears to function not only in thermal dissipation in light via xanthophyll pigments, but also modified the allocation pattern of excitation energy absorbed in the PSII to PSII photochemistry (P), thermal dissipation in light (DL) and excess excitation (E).

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